NTP
National Toxicology Program

Toxicology and Carcinogenesis Studies of Methylene Blue Trihydrate in F344 Rats and B6C3F1 Mice

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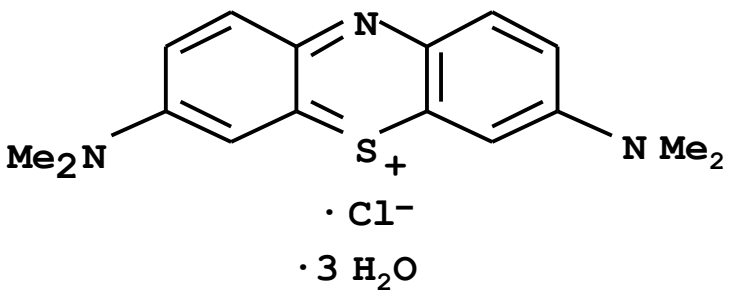
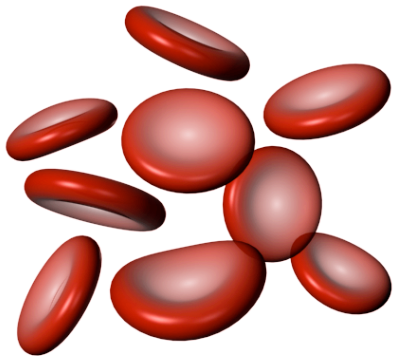
Methylene blue trihydrate



- Nominated by NCI because MB has numerous human and veterinary uses and lack of epidemiology and chronic toxicity studies.
- Uses: water-soluble biological stain, redox indicator, antimicrobial & antiseptic, methemoglobinemia, psychiatry, photodynamic chemotherapy, staging lymphoma
- NTP studies included:
  - rodent toxicity: 1-month, 3-month, and 2-year exposures, by gavage in 5% aqueous methyl cellulose, 5 days/week.
  - Genetic toxicity: *S. typhimurium & E. coli*, SCE & CA in CHO cells, MN in bone & blood
  - Neurotoxicity and reproductive toxicity: no effects
- MBT grade: >91%, highest purity available, similar to or exceeds purity of pharmaceutical grade used for human & veterinary therapies

Hematotoxicity of MBT



Methemoglobinemia & Heinz bodies

↓

Hemolysis

↓

Anemia &

Pigmentation (spleen, bone marrow, liver)

↓

Hematopoiesis (bone marrow and spleen)

1-Month Experiments in Rats and Mice

Groups: vehicle control & 5 exposed at 125, 250, 500, 1,000, 2,000 mg/kg

- **Mortality:**
  - **Rats - 2 males & 4 females 500 mg/kg, all higher doses**
  - **Mice - 2 males & 2 females 250 mg/kg, all at higher doses**
- **Body weights of surviving groups:**
  - **Rats - stat. sig. decreases at 250 mg/kg**
  - **Mice - similar to control**
- **Hematotoxicity:**
  - **Primary erythrocyte damage: methemoglobinemia & Heinz body formation.**
  - **Secondary observations reflect hemolysis and regenerative anemia**

High dose for 3-month range-finding study was selected to be lower than where deaths occurred in 1-month study.

3-Month Experiments in Rats and Mice

Groups: vehicle control & 4 exposed at 25, 50, 100, 200 mg/kg

- Mortality: FR only, 1/10 at 100 mg/kg and 4/10 at HD
- Group body weights: similar to control, except HD male rats*
- Spleen weight increase; only organ weight that correlated with dose
- Hematotoxicity indicated by hematology, clinical-pathology, anatomic-pathology [spleen & bone marrow], and organ-weight [spleen] effects.

Hematological effect	Rats	Mice
methemoglobinemia	≥ 50 mg/kg	≥ 25 mg/kg
Heinz body formation	≥ 100 mg/kg	≥ 25 mg/kg
regenerative anemia	≥ 50 mg/kg	≥ 25 mg/kg

* p=0.05 male rats.

Dose Selection for 2-Year Study

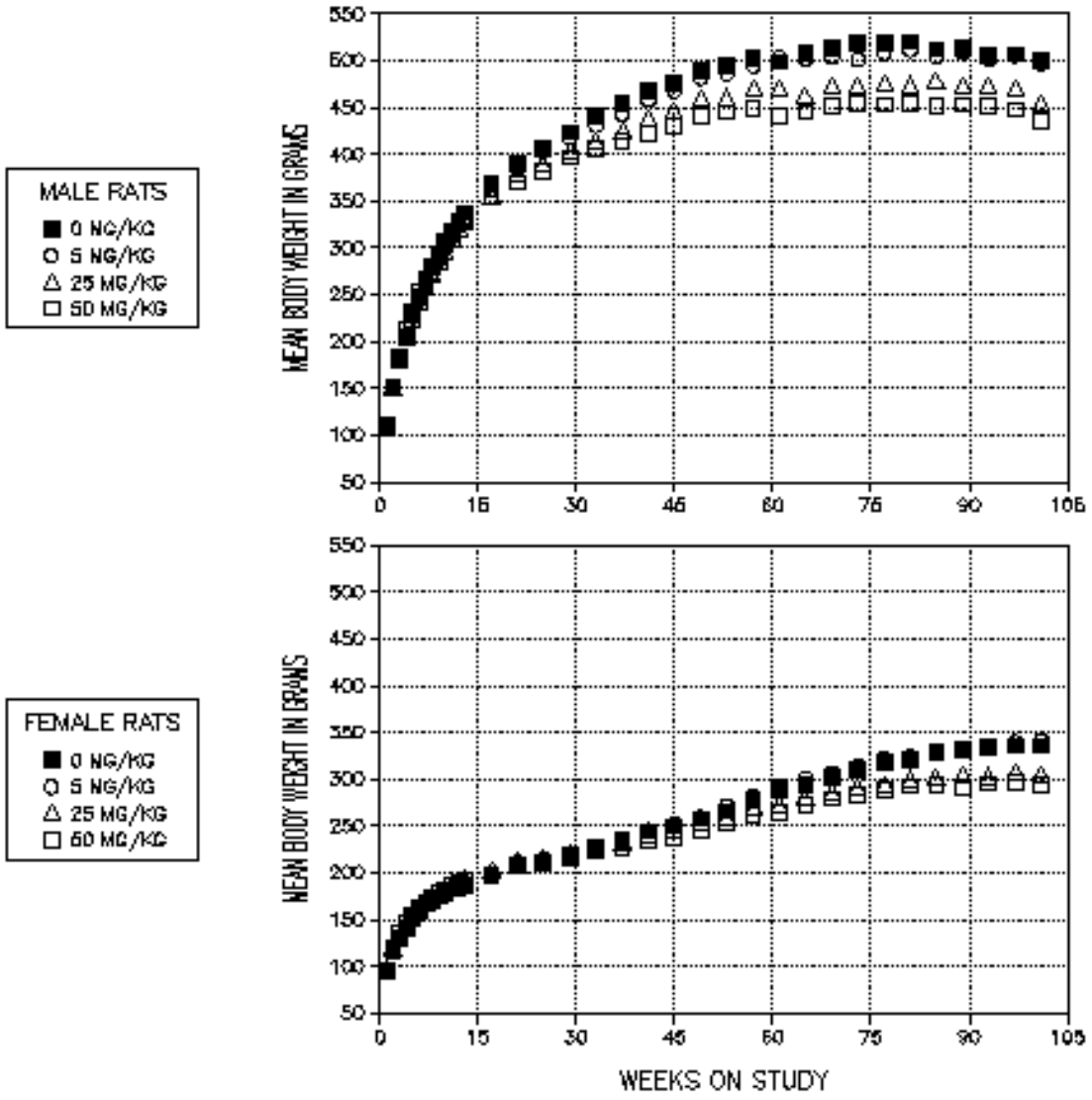
- **Selection rationale applies to both rats and mice:**
  - Highest dose was based on exposure level in 3-month study that gave minimal macrocytic, regenerative anemia
  - Mid dose was 1/2 high dose
  - Lowest dose 1/10 of high dose & similar to human therapeutic dose for methemoglobinemia, 1 to 2 mg/kg

- **Doses selected**

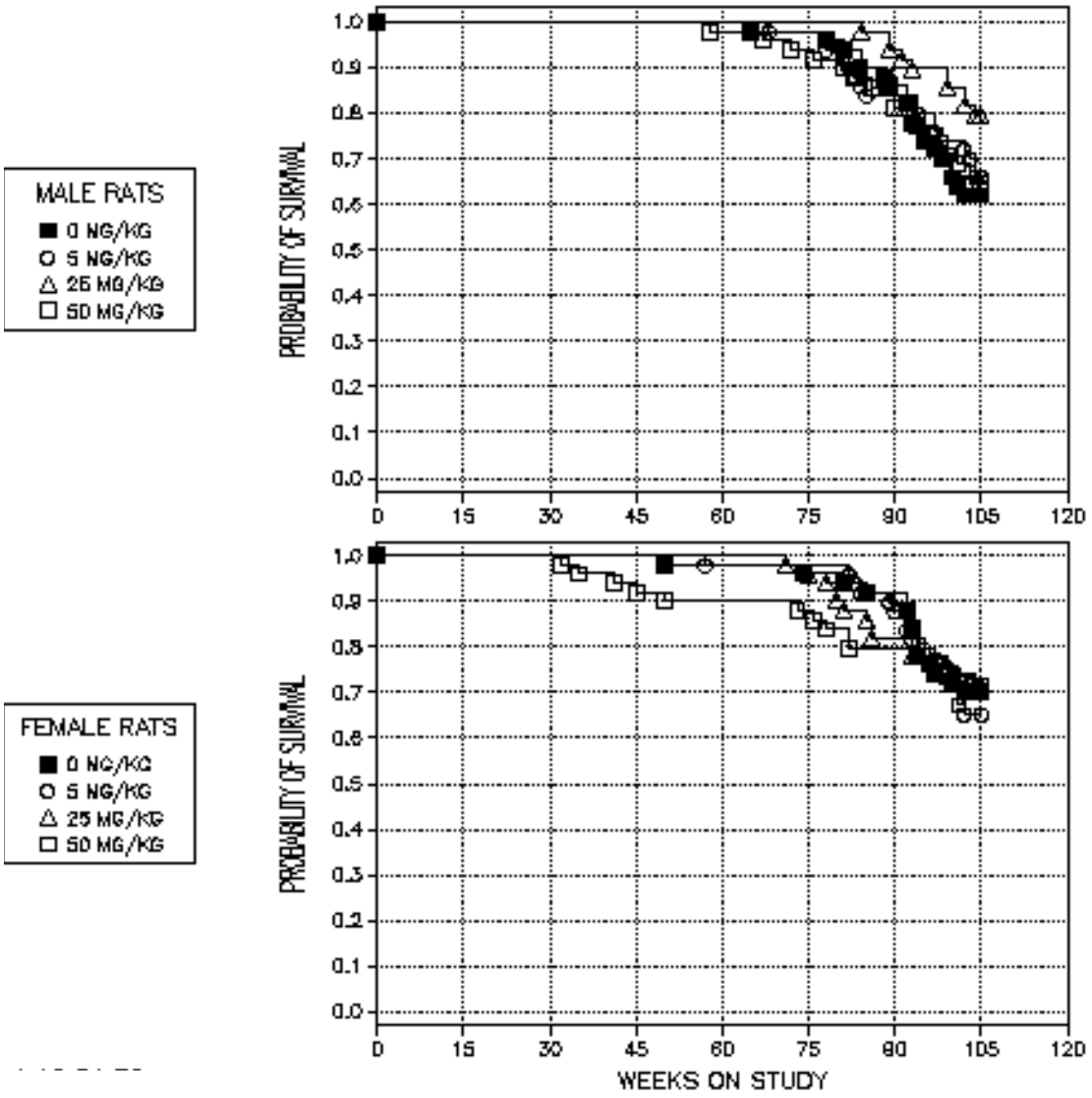
mg/kg by gavage[a]	vehicle control	low	mid	high
Rats, male and female	0	5	25	50
Mice, male and female	0	2.5	12.5	25

[a] In 5% aqueous methyl cellulose

Body-Weight Growth Curves, 2-Year Rats



Kaplan-Meier Estimated 2-Year Survival of Rats



Selected Nonneoplastic Lesions in 2-Year Rats

Dose, mg/kg:	0	5	25	50
Male pancreas[a]				
Acinus, focal hyperplasia	4	6	15**	12*
Atrophy	43	31**	35*	32*
Male spleen[a]				
Capsular fibrosis	1	7*	12**	30**
Hematopoietic cell prolif.	11	12	17	20*
Female spleen[b]				
Capsular fibrosis	8	17*	12	20**
Hematopoietic cell prolif.	3	5	7	8

[a] Male rats with lesion/50 examined microscopically

[b] Denominators for female rat groups: 49, 48, 49, 49

* $p \leq 0.05$ and ** $p \leq 0.01$ by Poly-3 test

Decreased Mononuclear Cell Leukemia in Rats

Dose, mg/kg:	0	5	25	50
Male, overall rate[a]	23 (46)**	10 (20)**	2 (4)**	2 (4)**
Female, overall rate	12 (24)**	6 (12) [b]	3 (6)*	2 (4)**

[a] Animals with lesion/50 examined, followed by rate %

[b] 49 animals examined

* $p \leq 0.05$ and ** $p \leq 0.01$ by Poly-3 test

- Dramatic decreases in MCL are result of inhibition caused by chronic toxicity to spleen, e.g. capsular fibrosis, secondary to hematotoxicity - Hall, 1990; Stefanski, *et al.*, 1990; Elwell *et al.*, 1996
- Other significant decreases:
  - FR, Mammary gland hyperplasia and fibroadenoma
  - MR, Pheochromocytoma(s) of adrenal medulla

Pancreatic Islet Cell Lesions in Male Rats

Dose, mg/kg:	0	5	25	50
Hyperplasia[a]	13	13	17	26**
Adenoma[b, c]	4 (8)	9 (18)	12 (24)*	8 (16)
Carcinoma	0	0	2	0
Adenoma or carcinoma[d]	4 (8)	9 (18)	14 (28)*	8 (16)

[a] Animals with lesion/50 examined

[b] Animals with lesion/50 examined, followed by rate %

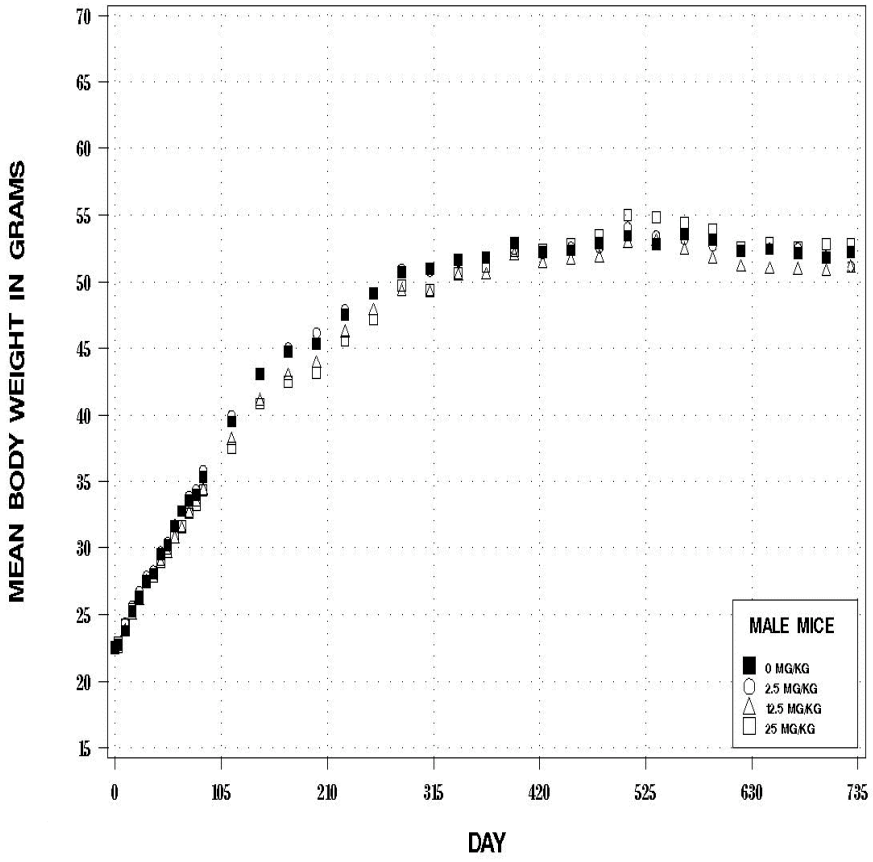
[c] Historical rate, adenoma = 66/1,448 (4.8 ± 3.1%), range = 0-10%

[d] Historical rate, combined = 92/1,448 (6.8 ± 4.4%), range = 0-14%

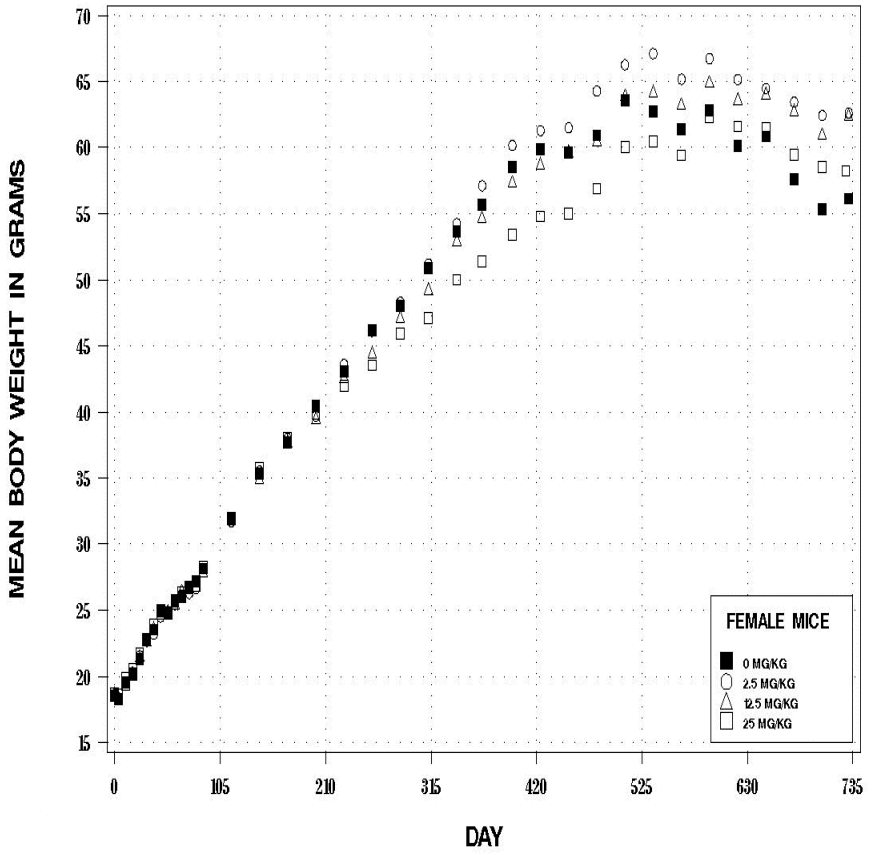
*$p \leq 0.05$ and **$p \leq 0.01$ by Poly 3 test.

2-Year Mice, Body-Weight Growth Curves

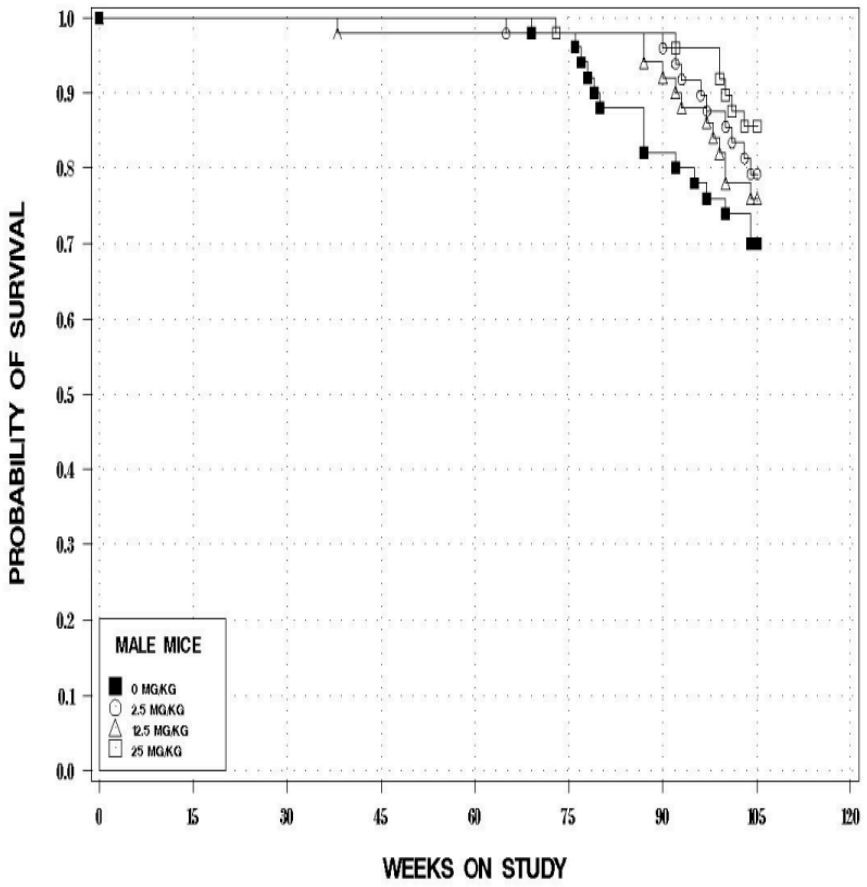
Male mice

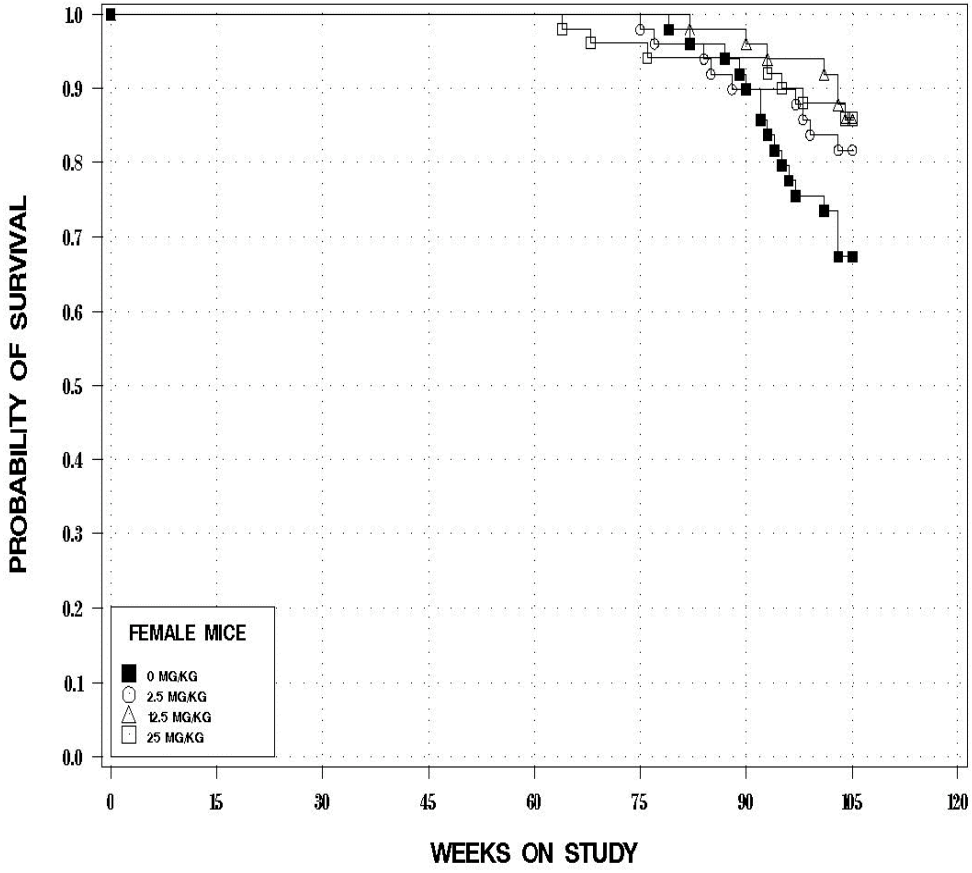


Female mice



Kaplan-Meier Estimated 2-Year Survival of Mice





Selected Nonneoplastic Lesions in Mice

Dose, mg/kg:	0	2.5	12.5	25
Spleen, hematopoietic cell proliferation				
MM:	14/49[a]	16/50	25/49*	29/48**
FM:	23/47	21/47	31/49	40/49**
Nose, inflammation				
MM:	1/50	3/50	3/50	6/50
FM:	0/50	3/50	7/50*	11/50**

[a] Animals with lesion/50 examined microscopically

* $p \leq 0.05$ or ** $p \leq 0.01$ vs. control group by the Poly-3 test

Tumors in Small Intestine of Male Mice

Dose, mg/kg	0	2.5	12.5	25
Adenoma	1[a] (2)[b]	1 (2)	2 (4)	2 (4)
Carcinoma[c]	0 (0)*	1 (2)	2 (4)	4 (8)*
Adenoma or carcinoma[d]	1 (2)*	2 (4)	4 (8)	6 (12)*

[a] Animals with lesion/50 with tissue examined at necropsy

[b] Incidence as rate %

[c] Historical rate, gavage: 8/249 (3.2%), range = 0-8%
all routes & vehicles: 33/1,508 (2.2% ± 2.7%), range = 0-10%

[d] Historical rate, gavage: 10/249 (4.0%), range = 0-8%
all routes & vehicles: 39/1,508 (2.6% ± 2.8%), range = 0-10%

* $p \leq 0.05$ for trend or vs. control group by the Poly-3 test

Malignant Lymphomas in Mice

Dose, mg/kg	0	5	25	50
Male[a, b]	2 (4)	2 (4)	2 (4)	5 (10)
Female[c]	6 (12)*	4 (8)	9 (18)	12 (24)

[a] Animals with lesion/50 examined microscopically, followed by rate %

[b] Male historical rate, gavage: 13/249 (5.2%), range = 4-6%
all routes & vehicles: 70/1,508 (4.3% ± 2.3%), range = 0-8%

[c] Female historical rate, gavage: 31/250 (12.4%), range = 8-18%
all routes & vehicles: 308/1,558 (19.7% ± 13.3%), range = 6-58%

* $p \leq 0.05$ for trend by the Poly-3 test

Genetic Toxicity Test Results for Methylene Blue Trihydrate

Test type	Endpoint	Results[a]
In vitro*		
Ames test	Bacterial mutagenicity	+/+
CHO-cell chromosome damage assays	Sister chromatid exchanges	+/+
	Chromosomal aberrations	+/+
In vivo		
Acute micronucleus (MN) test in male mice	MN reticulocytes in bone marrow and blood	-
Subchronic MN test in male and female mice	MN erythrocytes in blood	-/-

* With and without metabolic activation by rat or hamster S9 liver enzymes.
[a] +, positive; -, negative

Levels of evidence for carcinogenic activity of MBT in male and female rats and mice

Some evidence in male rats based on increased incidences of adenoma and of adenoma or carcinoma [combined] in **pancreatic islet cells**.

No evidence in female rats exposed to 5, 25, or 50 mg/kg.

Some evidence in male mice based on increased incidences of carcinoma and of adenoma or carcinoma [combined] in **small intestine**. **Malignant lymphoma** may have been related to MBT exposure.

Equivocal evidence in female mice based on increasing trend of **malignant lymphoma** and incidences that exceeded mean rate and range for historical controls.

Decreases in mononuclear cell leukemia in male and female rats were related to MBT exposure**.**